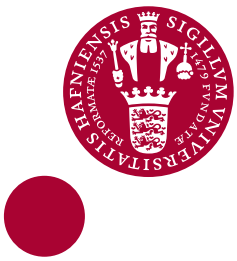

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Brandt, Kristian Koefoed; Holm, Peter Engelund; Nybroe, Ole

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Bioavailability of soil particle-bound copper to *lux*-marked, copper-specific *Pseudomonas fluorescens* biosensor

Kristian K. Brandt[2], Peter E. Holm[1], Ole Nybroe[2]

[1]Department of Natural Sciences (peho@kvl.dk); [2]Department of Ecology (kkb@kvl.dk - oln@kvl.dk), The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, DENMARK.

INTRODUCTION

The measurement of metal bioavailability to soil dwelling organisms remains a challenge. Usually, only the soluble fraction of metals is considered bioavailable and thus able to exert a physiological response, but this has rarely been tested experimentally. Interestingly however, it has very recently been reported that a significant fraction of particle-bound metals such as cadmium and lead are bioavailable to specific bacterial whole-cell biosensors in soil-water mixtures (Ivask et al. 2004). Hence, up to 115-times more Cd and 40-times more Pb proved bioavailable to sensor bacteria incubated in soil suspensions rather than in aqueous soil extracts (Ivask et al. 2004). These somewhat surprising results highlight the need for further research in this field.

The aim of the present work was to develop a solid phase-contact bioassay based on a Cu-specific, *luxAB*-marked *Pseudomonas fluorescens* reporter bacterium allowing for the direct interaction between sensor cells and particle-bound Cu in soil and to evaluate the bioavailability of particle-bound Cu under different experimental conditions.

METHODS

A sandy loam containing 10 ppm Cu was sampled, partially dried, sieved and divided into 2 fractions. One fraction was added dissolved $CuSO_4$ to a final concentration of 160 ppm and water to a soil moisture content of 15% wt/wt (~60% of water-holding capacity). The other fraction (control soil) was added water only. The soils were stored at 5°C for 4-5 months.

A Cu-specific, bioluminescent reporter strain (*Pseudomonas fluorescens* DF57-Cu15) carrying a chromosomal insertion of a promoter-less Tn*5::luxAB* cassette controlled by a Cu-induced promoter was used to estimate the bioavailable concentration of Cu (Tom-Petersen et al. 2001 and 2004). Another almost identical strain (*P. fluorescens* DF57-40E7), showing a constitutive expression of the *luxAB* gene cassette was used in parallel to correct for sample matrix interference with sensor bacteria due to the presence of substrates (stimulation), toxicants (inhibition) and soil particles (quenching of luminescence).

P. fluorescens DF57-Cu15/40E7 cultures were harvested in the exponential growth phase and reporter assays conducted as follows: Cell suspension was mixed with sample or Cu standard and incubated for 90 min before bacterial bioluminescence was measured. Samples consisted of either soil-water mixtures (slurries) or the corresponding particle-free soil extracts prepared by centrifugation of soil slurries (10000 × g, 5 min).

Bioluminescence data was backed by determinations of total soil Cu and total dissolved Cu using Graphite Furnace AAS. Soil samples were subject to total digestion before analysis.

RESULTS AND DISCUSSION

An overview of the obtained results is presented in Table 1. The bioavailable pool of Cu in particle-free soil extracts only accounted for 0.23 % of the total Cu content of the Cu-spiked soil. When measurements were performed in soil-water mixtures, this figure increased up to 86-fold, as up to 20 % of the total Cu pool was available in soil slurries diluted with Milli-Q

water. This observation is in line with previous reports based on data from similar solid phase-contact bioassays (Ivask et al. 2004), but our data suggest that the increased bioavailability in dilute soil-water mixtures may be caused by an artefact related to the low soil-water ratio used. Hence, when soil slurry samples were diluted with 'Cu-free' soil slurry containing negligible bioavailable Cu, only 0.31 % of the total soil Cu content were available to the sensor cells, indicating that particle-bound Cu is not directly available to *P. fluorescence* in soil.

Table 1. Cu bioavailability estimates based on bioluminescence measurements.

Soil	Sensor bacteria incubated in	Dilution factor	Soil-water ratio	Sample diluted with	Bioavailability (% of total Cu)	*n*
Control soil	Soil-water mixture	1	1:10	-	b.d.	3
Cu-spiked soil	Soil-water mixture	1	1:10	-	> 0.06 [a.d.]	3
Cu-spiked soil	Soil-water mixture	10	1:100	Milli Q water	> 0.62 [a.d.]	3
Cu-spiked soil	Soil-water mixture	100	1:1000	Milli Q water	9.46 ± 2.32	3
Cu-spiked soil	Soil-water mixture	1000	1:10000	Milli Q water	19.70 ± 6.40	3
Cu-spiked soil	Soil-water mixture	10	1:10	Control soil slurry	0.31 ± 0.09	4
Cu-spiked soil	Soil-water mixture	100	1:10	Control soil slurry	0.84 ± 0.40	4
Cu-spiked soil	Soil-water mixture	1000	1:10	Control soil slurry	b.d.	2
Cu-spiked soil	Particle-free extract [a]	-	-	Milli Q water	0.23 ± 0.09 [d]	3
Cu-spiked soil	Particle-free extract [b]	-	-	Milli Q water	0.80 ± 0.12 [d]	3
Cu-spiked soil	Particle-free extract [c]	-	-	Milli Q water	1.89 ± 0.23 [d]	3

[b.d.] below detection limit (i.e. *luxAB* expression in Cu-specific biosensor is not induced).
[a.d.] above detection limit (i.e. Cu is toxic to sensor bacteria).
[a] Soil particle-free extract prepared from soil-water mixture having a soil-water ratio of 1:10.
[b] Soil particle-free extract prepared from soil-water mixture having a soil-water ratio of 1:100.
[c] Soil particle-free extract prepared from soil-water mixture having a soil-water ratio of 1:1000.
[d] Numbers refer to % of total Cu in soils from which particle-free extracts were derived. Usually, 20-100 % of total dissolved Cu in soil extracts is available to sensor bacteria (Tom-Petersen et al. 2004; Brandt et al., unpublished results).

CONCLUSIONS

- The *P. fluorescens* solid phase-contact assay provides a sensitive estimate of bioavailable Cu in soil-water mixtures.
- Particle-bound Cu is not directly available to *P. fluorescence* in soil slurries having a high soil-to-water ratio (1:10).
- Our data supports the conventional view that only the soluble fraction of metals are bioavailable in soil and calls for a critical re-evaluation of recent reports claiming that particle-bound metals are available to bacteria in soil.

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